Colonisation of Pine and Spruce Poles by Soil Fungi after Six Months

By AINO KÄÄRIK*

Royal College of Forestry, Stockholm (Sweden)

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1. Introduction

Poles of pine and spruce not treated with preservatives are, if partly buried in soil, usually very soon invaded by fungi and decay rapidly. Marked differences in the rate of decay exist on different sample plots, apparently due to different climatic and soil conditions. T. C. SCHEFFER (1964) recognised differences in fungal attack at different places in one sample plot, which resulted from the unequal distribution of decay fungi. The later stages of decay in such wood material are usually associated with basidiomycetous fungi, although some Ascomycetes and Fungi imperfecti are concerned, too. Information about the fungi concerned in the early stages of decay in such material, as also about the place and rate of invasion and the fungal succession on different sites, is scarce. W. MERRILL and D. W. FRENCH (1966) recently investigated the colonisation by fungi of Ponderosa pine stakes during four summer months. They found that there was already a considerable number of fungi, especially Phycomycetes and Fungi imperfecti, after some weeks' exposure. H. GREAVES and J. G. SAVORY (1965) record a number of non-basidiomycetous fungi from preservative-treated wood in contact with the soil. Several of these fungi have been shown to be able to utilise or decompose preservatives (C. M. CHRISTENSEN, F. H. KAUFERT, H. SCHMITZ and J. L. ALLISEN, 1942; C. MADHOSINGH, 1961; C. G. DUNCAN and F. J.

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* Dedicated to Dr. GERDA THEDEN on the occasion of her 60th birthday.

DEVERALL, 1964). Since the fungi which occur on wood not only attack it in different ways, but also react very differently to preservatives, it is of interest to know which fungi occur normally with great frequency on wood in contact with the soil on different sites, their ability to decompose wood and their reaction to preservatives. In the present investigation the colonisation by fungi of pine and spruce poles in contact with the soil in Simlångsdalen (South Sweden) on three different soil types was studied. A list of fungi colonising such wood after six months is presented here.

2. Materials and methods

Test poles, 10—12 cm in diameter and 120 cm long, from freshly cut young pine and spruce stems were barked in the vicinity of sample plots and driven vertically into the soil to a depth of 60 cm, about one metre from each other on three different sample plots. After six months (May 15th to November 15th) two poles of each tree species were taken up on every plot; the soil was wiped off and disks 1.5 cm thick were sawn out immediately at five different levels, wrapped in plastic bags and transported to the local laboratory. The disks were so marked that the separate pieces removed for culturing from every disk were kept in the same order as they had occupied relative to the stem's long axis. On the same day, samples for culturing were removed with a sterile knife from every disk. In the laboratory, 12 samples (ca. 6 mm × 6 mm × 15 mm) were removed from each disk, distributed evenly along two diameters crossing each other at right-angles. These samples were dipped briefly in alcohol, flamed, split again radially on a sterile substratum with a flamed knife and from this new wood surface five small (about 1 mm × 1 mm × 1—2 mm) pieces were taken with sterile forceps and placed on malt agar in Petri dishes. The tools were sterilised between each isolation. The plates were incubated at room temperature, examined after five to seven days, then after two weeks; the fungi which had grown out from the pieces were transplanted on to new dishes if they were not identified directly. Thus, from each pole 300 pieces were removed, making 3600 pieces altogether.

Before the use of malt extract agar medium (2.5 per cent malt extract, 1.5 per cent agar) was adopted, different substrates were tried for the isolation of fungi from similar material, but none of them showed better results than malt extract agar. The following substrates were tested: weak malt agar, acidified malt agar, Kuhlman agar, containing streptomycin and PCNB (E. G. KUHLMAN and F. F. HENDRIX, 1962), starch-salt agar, cellulose-salt agar, sawdust agar and malt extract agar with the addition of induline (1 g), resorcin (1 g). gentian violet (0.07 g), malachite green (0.07 g), merthiosal (0.017 g) or α-naphthol (0.07 g) per 1000 ml solution respectively. Bacteria were found in many samples, but they did not inhibit the growth of the fungus mycelia.

The three sample plots were situated as follows: 1) in a wet birch-alder carr near the river Fylleån with *Monilia coerulea* — *Vaccinium vitis-idaea* community, the soil being water-logged early in the spring and late in the autumn 2) in a spruce stand with *Deschampsia flexuosa* — *Oxalis acetosella* community on moderately moist sandy soil and glacial drift and 3) in a beech stand with *Deschampsia flexuosa* — *Luzula pilosa* community on rather dry morainal gravel (cf. C. MALMSTRÖM, 1937). The method adapted after A. L. SHIGO (1965), viz. plating out very small pieces of wood immediately after sectioning the trees, worked well with the present material. Practically no infection with the common moulds occurred, whereas methods using larger blocks of wood or aseptically taken increment borer cores were not satisfactory for use when cultivating fungi from wood in contact with the soil, the samples showing much contamination.

3. Results

After six months, the poles, except for those standing in the wet soil, were heavily infected by blueing fungi and clear patches of decay were visible at almost all levels in the poles (tab. 1). In the tables 1—4 the sections of the poles are enumerated as follows: 1. 2 cm from the lower end of the pole, 60 cm beneath the soil; 2. 30 cm beneath the soil; 3. soil surface; 4. 30 cm above the soil; and 5. 60 cm above the soil, 2 cm from the upper end of the pole. In tables 2—4 the samples in column A were taken from outer sapwood, B from inner sapwood and C from heartwood, the columns A, B and C representing 40 samples each.

Tab. 1. Estimated area of the sections colonised by decay and blue stain fungi after six months. Sections taken from spruce and pine poles at different levels. Invaded area in per cent of the section.

Section no.	birch carr				spruce stand				beech stand			
	spruce		pine		spruce		pine		spruce		pine	
	decay	blue st.	decay	blue st.	decay	blue st.	decay	blue st.	decay	blue st.	decay	blue st.
1	—	40	—	50	5	80	5	80	—	60	—	60
2	—	50	—	60	5	60	5	60	5	50	5	50
3	—	80	—	90	10	50	10	30	10	50	10	50
4	10	80	5	90	15	50	10	30	10	50	10	30
5	15	80	10	80	20	90	15	60	10	80	15	60

The identification of the mycelia isolated is preliminary in the case of some Fungi imperfecti.

Tables 2, 3 and 4 represent the distribution of fungi in the poles at different levels and in different layers of the wood. A surprisingly large number of fungi was found, some of them growing through the whole stem, others occurring in more restricted areas. In tables 2 and 3 the fungi occurring on two pine and spruce poles in the spruce stand are

T a b. 2. Fungi isolated from spruce poles in a spruce stand from different levels and from different layers of the wood.

Fungi isolated	Section no.														
	1			2			3			4			5		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Basidiomycetes															
Corticium laeve														5	
Fomes annosus							3								
Hypholoma fasciculare		2				1									
Peniophora pithya													28	10	4
Polyporus borealis							1								
Polyporus caesius											1	1			
Stereum abietinum										7	6				
Stereum sanguinolentum													4	7	
Basidiomycete sp.		1					2	2		2	1				
Other mycelia															
Alternaria humicola													3	4	3
Botrytis cinerea													1		
Ceratocystis piceae	7			6	6	3	18	26	18		6		3	2	
Ceratocystis sp.				2	2										
Chaetomium sp.				3						5	4	12	5	4	5
Cladosporium herbarum							3		2	2		1			
Cladosporium macrocarpum							4			2	6	5	6	5	
Coniochaeta ligniaria													1		
Cytospora sp.		3													
Cytosporella sp.										7					
Discula pinicola				1											
Fusarium solani														1	1
Haplographium penicilloides						1									
Monosporium olivaceum		6	3	3											
Mucor sp.															1
Oidiodendron tenuissimum											2	2		1	1
Orbicula sp. (cf. *parietina*)												1		2	2
Pachybasium sp.	2	6		4	10	6		1			1				
Pestalotia funerea				4	4	4									
Phialophora aurantiaca	1	1		4	5	4	2		6				5	2	2
Phialophora lutea-olivacea						3									
Pullularia pullulans									1						
Rhinocladiella sp.				2		1									
Sclerophoma pityophila											2				
Torula sp.														2	
Trichocladium sp.		5	2	2	3		10	4		8	6		6	1	
Trichoderma spp.	15	12	15	8	8	8	10	4	3	1	1				
Blue stain sp. (A)	1	3	2	3	2	2		2		2					
Blue stain sp. (C)				1		2			1						
Hyaline spp.	7	2	3	4	7					8	1		10	5	9
Number of fungi in 40 samples (each column)	33	41	26	47	47	35	53	39	31	44	37	22	72	51	28

T a b. 3. Fungi isolated from pine poles in a spruce stand from different levels and from different layers of the wood.

Fungi isolated	Section no.														
	1			2			3			4			5		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Basidiomycetes															
Odontia bicolor		2													
Pleurotus mitis							2	2							
Poria cinerascens		2	6			7									
Poria rixosa	4	2													
Poria versipora							3	2	8						
Stereum sanguinolentum										2	2		3		
Trametes serialis													5	2	
Basidiomycete sp. (A)									10				5	8	6
Basidiomycete sp. (B)				3											
Basidiomycete sp. (C)		1											2	4	
Other mycelia															
Botrytis cinerea															4
Ceratocystis piceae	1	8		1	5	1	13	14	2	1					
Ceratocystis pilifera							4	9	3						
Chaetomium sp. (B)										25	7		8	8	3
Cladosporium herbarum		1													
Diplodia sp.									2						
Discula pinicola										10	3	2		12	
Fusarium solani								1							1
Monosporium olivaceum		3													
Mucor sp.	1														
Oidiodendron tenuissimum						2									
Orbicula sp.			2	1	5			2	1	1	1				
Pachybasium sp.	12	11	4	4	3	1	11	7	4			2			2
Phialophora lutea-olivacea	3	1	1		2					1	1				
Phoma sp.					1										
Pullularia pullulans										2	1	1			
Rhinocladiella sp.			4												
Sclerophoma pityophila													14	4	22
Torula sp.															2
Trichoderma sp.	14	7	6	23	11	7	13	15	12	7	4	1	6	2	
Verticicladiella sp.		1	8	4	3	16		4							
Blue stain sp. (C)	8	8	10	6	5	7									
Blue stain spp.							11	1	11	2	4	1	1		3
Hyaline spp.	7	15	4	10	1		2		4	3		2	2	6	
Number of fungi in 40 samples (each column)	50	62	45	52	36	43	59	57	57	54	23	9	45	44	43

Tab. 4. Decay fungi isolated from spruce and pine poles in the birch carr and beech stands.

Fungi isolated	Section no.														
	1			2			3			4			5		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Birch carr, spruce															
Fomes nigrolimitatus												2			
Stereum sanguinolentum												5			
Trechispora brinkmanni														3	5
Basidiomycete sp. (D)									2						6
Polyporus fibrillosus								1							
Basidiomycete sp.						1									
Birch carr, pine															
Polyporus amorphus										3	17		1		
Stereum sanguinolentum										11	14	4	5	4	1
Basidiomycete sp. (A)										1	2	3		3	5
Basidiomycete sp.	1														
Beech stand, spruce															
Corticium laeve							2	3					2	3	
Corticium sp. (F)				4			3		5			1			
Fomes annosus				4	7										
Peniophora pithya			1	1		10	5	5							
Stereum sanguinolentum										3	2				
Trechispora sp.													5	1	
Basidiomycete sp.						6			5			1	5	6	
Beech stand, pine															
Fomes nigrolimitatus													3	4	
Phlebia radiata															4
Polyporus benzoinus										7	3		2	1	
Poria rixosa	10	8	3		1		6								
Stereum sanguinolentum								1		4					
Basidiomycete spp.	7	8	6			10		3	3	5	5		4	7	5
Each column in each locality representing 40 samples.															

Tab. 5. Frequency of the decay fungi on all test plots.

Fungi isolated	Locality and tree species					
	birch carr		spruce stand		beech stand	
	spruce	pine	spruce	pine	spruce	pine
Corticium laeve			5		10	
Corticium sp. (F)					13	
Fomes annosus			3		11	
Fomes nigrolimitatus	2					7
Hypholoma fasciculare			3			
Odontia bicolor				2		
Peniophora pithya			42		22	
Phlebia radiata						4
Pleurotus mitis				4		
Polyporus amorphus		21				
Polyporus benzoinus						13
Polyporus borealis			1			
Polyporus caesius			2			
Polyporus fibrillosus	1					
Poria cinerascens				15		
Poria rixosa				6		28
Poria versipora				13		
Stereum abietinum			13			
Stereum sanguinolentum	5	39	11	7	5	5
Trametes serialis				7		
Trechispora brinkmanni	8				6	
Basidiomycete sp. (A)		14		29		
Basidiomycete sp. (B)				3		
Basidiomycete sp. (C)				7		
Basidiomycete sp. (D)	1					
Basidiomycete spp.	8	1	8		23	63
Number of fungi in 600 samples (each column)	25	75	88	93	90	120

Tab. 6. Frequency of Asomycetes and Fungi imperfecti on all test plots.

Frequency of mycelia	Locality and tree species					
	birch carr		spruce stand		beech stand	
	spruce	pine	spruce	pine	spruce	pine
Alternaria humicola	—	—	++	—	+	—
Botrytis cinerea	—	(+)	(+)	(+)	+	+
Ceratocystis piceae	++	+++++	++++	++++	+++++	+++++
Ceratocystis pilifera	(+)	++	—	++	—	+ (+)
Ceratocystis spp.	—	++	++	—	++	+
Chaetomium sp. (A)	—	—	++	—	—	—
Chaetomium sp. (B)	—	—	++++	+++	—	—
Cladosporium herbarum	+	+	+++	(+)	+	(+)
Cladosporium macrocarpum	(+)	—	+++	—	+++	+++
Cylindrocarpon radicicola	+++	—	—	—	+	++
Cytospora sp.	+++	+++	+	—	—	—
Discula pinicola	+++++	++++	(+)	+++	+++	++
Fusarium solani	+++	++	+	+	—	—
Fusarium spp.	—	—	—	—	+++	+++
Monosporium olivaceum	—	—	++	—	++	—
Mucor sp.	—	+	(+)	(+)	—	—
Oidiodendron tenuissimum	+	—	++	(+)	—	(+)
Orbicula sp.	—	++	++	+++	(+)	+
Pachybasium sp.	+++	++++	+++	++++	+++	+++
Pestalotia funerea	+++++	+++	++	—	++	—
Phialophora aurantiaca	+	—	++++	—	+++	++
Phialophora lutea-olivacea	(+)	++	(+)	+++	—	+++
Phoma spp.	—	—	—	(+)	++	—
Pullularia pullulans	+	—	(+)	++	(+)	+++
Rhinocladiella sp.	—	+	+	+	—	—
Sclerophoma pityophila	—	++	(+)	++	++++	++++
Tilachlidium sp.	++	—	(+)	—	++	++
Torula sp.	—	+	(+)	(+)	—	++
Trichocladium sp.	+++	+++	+++++	+	++++	++++
Trichoderma sp.	++++	+++++	+++++	+++++	+++++	+++++
Verticicladiella spp.	(+)	+	—	++++	(+)	(+)
Blue stain sp. (A)	+++	++	++++	—	—	++
Blue stain sp. (B)	(+)	+++	++	+++	(+)	—
Blue stain spp.			—	+++	+++	+++
Hyaline spp.	++	++++	++++	++++	++++	+++

shown. On other sample plots, with the exception of Basidiomycetes, the fungus flora was rather similar; therefore only the Basidiomycetes are shown separately for the other sample plots (tab. 4), while the occurrence of other more frequent mycelia is summarised in tab. 6. A rather large number of Basidiomycetes was isolated from the poles; these mycelia were rather different on different sites (tab. 4 and 5). Numerous fungi occurred in one pole and most of them occurred in rather small patches. Some of them, such as *Poria rixosa, Fomes annosus, Odontia bicolor* and *Peniophora pithya,* were found mainly beneath the soil, whereas others, such as *Stereum sanguinolentum, Pleurotus mitis* and *Polyporus amorphus* occurred mainly above the soil. In the samples from the wet carr no decay fungi were found beneath the soil, but the other fungi were almost as numerous beneath the soil as above it. Many basidiomycete mycelia were not identified. These belong in all probability to Thelephoraceae not yet known in culture. Some of these fungi had rather well characterised mycelia. In tab. 6, fungi occurring only sporadically on one pole are not included. Such fungi were: *Coniochaeta ligniaria, Coryne sarcoides, Curvularia lunata, Cylindrocephalum stellatum, Diplodia* sp., *Fusarium oxysporum, Geomyces vulgaris, Gliocladium viride, Haplographium penicilloides, Oidiodendron griseum, Paecilomyces variotii, Phialophora fastigiata* and *Spicaria* sp. In the wet plot, *Pestalotia funerea* was a dominant fungus in spruce wood at all levels and in all layers of the wood. In pine wood it was quite common, but only beneath the soil. Another very frequent fungus was *Discula pinicola,* occurring on both pine and spruce. The frequent occurrence of *Ceratocystis piceae* on pine can be explained by the attack of *Xyloterus lineatus* on these poles.

4. Discussion

From the above studies it may be seen that on all three sample plots after six months the decay fungi had attacked the poles from several locally isolated points and that several Basidiomycetes were involved in the first stages of decay. The number of mycelia of both Ascomycetes, and especially Fungi imperfecti, colonising the poles was rather large, many of them occurring only locally on one pole, others growing over large areas or over the whole pole. Often several fungi were isolated from one minute piece of wood. It was noticeable that on the poles studied the otherwise very common, fast-growing and readily recognisable fungi such as *Polyporus abietinus* and *Peniophora gigantea* were not met once. These fungi are among the first Basidiomycetes to colonise fresh stumps in similar localities and are often very frequent there (A. Käärik and E. Rennerfelt, 1957). The flora of blue stain fungi on the poles, too, was totally different from that on the stumps if they were examined the same length of time after felling. W. Merrill and D. W. French (1966) found, after four months, quite different fungi, ycetes, *Penicillium* and *Aspergillus*. Among the dominating fungi they recognised *Fusarium solani* and *Trichoderma viride*, i. e. fungi which occurred frequently in the present material. In the material presented here, however, Phycomycetes were almost entirely absent, only a *Mucor* sp. occurring very sporadically in the top sections.

Of the non-basidiomycetous fungi occurring in the present material, several have been shown to decompose wood (C. G. Duncan, 1965; C. G. Duncan and W. W. Eslyn, 1966) under certain circumstances. Such fungi are *Cladosporium herbarum, Fusarium solani, Pestalotia funerea, Alternaria, Chaetomium, Cytospora, Cytosporella, Graphium, Monosporium, Orbicula, Phialophora* and *Phoma* spp. It may be of interest to follow the development of this quite rich fungus flora, consisting of various types of fungi, every one utilising the wood in its own way, during a longer period of time.

5. Summary

The colonisation by fungi of pine and spruce poles in contact with the soil was studied on three different soil types in South Sweden. Fungal mycelia were isolated from different levels and from different wood layers on the poles. After six months the poles showed attack by different Basidiomycetes on several locally restricted patches. The dominating decay fungi were different in the different stands, but all belonged to the common forest flora. Besides the decay fungi, numerous mycelia of Fungi imperfecti and Ascomycetes were isolated. Many of these were found on restricted patches in the poles, but some were growing throughout the pole. Several fungi were often growing out from minute samples. The fungus flora on all three sites was found to be rather similar, but quite marked differences in the frequency of a number of species was found in the different stands.

Zusammenfassung

Besiedelung von Kiefern- und Fichtenmasten durch Erdpilze nach sechs Monaten

Die Besiedelung in Erde eingebauter Kiefern- und Fichtenmaste durch Pilze wurde auf drei verschiedenen Boden-Typen in Südschweden untersucht. Pilzmycel wurde aus verschiedenen Mastbereichen und Holzschichten isoliert. Nach sechs Monaten zeigten die Maste Befall durch verschiedene Basidiomyceten an mehreren begrenzten Stellen. In den verschiedenen Beständen herrschten unterschiedliche Pilze vor. Sie gehörten jedoch alle der herkömmlichen Waldflora an. Außer den holzabbauenden Pilzen wurde noch Mycel mehrerer Fungi imperfecti und Ascomyceten isoliert. Viele fanden sich nur an begrenzten Stellen, einige durchwuchsen jedoch den ganzen Mast. Oft wuch-

sen mehrere Pilze aus sehr kleinen Proben. Die Pilzflora auf allen drei Böden war sich sehr ähnlich, obwohl die Häufigkeit einer Art in den verschiedenen Beständen sehr unterschiedlich war.

Résumé

Infestation au bout de six mois de poteaux de pin et de sapin par des champignons du sol

L'infestation par les champignons, de poteaux de pin et de sapin en contact avec le sol, a été étudiée dans trois types de sols différents de la Suède méridionale. Sur les poteaux, on a isolé du mycélium fongique à différents niveaux et dans diverses couches de bois. Après six mois, on a noté sur les poteaux, une attaque par divers Basidiomycètes sur des zones localement restreintes. Les moisissures dominantes étaient différentes dans les diverses stations mais appartenaient toutes à la flore forestière commune. En outre, ont été isolés de nombreux mycélia de Fungi imperfecti et d'Ascomycètes. Un grand nombre de ceux-ci furent trouvés dans des zones localisées des poteaux mais quelques uns se développaient dans toute la masse du poteau. Plusieurs champignons se développaient souvent à partir de très petits échantillons. La flore fongique était assez semblable dans les trois stations mais il y avait des différences marquées dans la fréquence d'un certain nombre d'espèces dans les diverses stations.

Resumen

Colonización por hongos de pinos y abetos en suelos después de seis meses

Se estudia en tres tipos diferentes de suelos del sur de suecia la colonización por hongos de pinos y abetos en contacto con el suelo. Fungal mycelia fueron aislados de diferentes niveles y de diferentes capas de la madera en los abetos. Después de seis meses los abetos mostraban ataques por diferentes Basidiomycetos en varias manchas locales. El predominante detorioro debido a los hongos fue distinto en los diferentes niveles pero todos pertenecen a una flora común del bosque. Se aislaron además del deterioro de hongos numerosas micelas de Fungi imperfecti y Ascomycetes. Muchos de estos fueron encontrados en manchas de los abetos aunque algunos crecían a través del abeto. Algunos hongos crecieron en pequeñas muestras. Se vió que la flora de hongos en los tres lugares indicados era más bien similar, pero existe una marcada diferencia en la frecuencia de un número de especies que se encontraron on otros niveles.

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Address of the author:
Dr. AINO KÄÄRIK
Royal College of Forestry
Stockholm 50
Sweden